COMPARATIVE DISTRIBUTION AND POSSIBLE PATHOGENICITY OF PARACOLOBACTRUM SPECIES IN AN AREA HIGHLY ENDEMIC FOR ENTERIC INFECTIONS

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In an area where enteric infections are highly endemic, the etiological agents of the infections are not always clearly evident. For example, the percentage of specimens positive for commonly accepted intestinal pathogens in over 800 stool specimens examined in North Africa from May through September, 1943, fell from 70 per cent during May and June, when gastroenteritis among American troops was at its maximum, to 30 per cent and then to 10 per cent in the months following. Inasmuch as the majority of specimens examined were from cases of enteritis, it was felt that the commonly accepted pathogens were not always the cause of these disorders, and it was decided to investigate the comparative distribution of Paracolobactrum species. Such a study, it was believed, would yield additional information concerning possible pathogenicity of that group of organisms and would, in addition, furnish data on their relative frequency in normal individuals in an endemic area. The investigation was therefore initiated in January, 1944, and specimens were collected until May of that year in Casablanca, North Africa, the cultural and serological work being continued until August, 1944, following transfer of the organization to Rome, Italy. In this paper, the nomenclature for the paracolon organisms proposed by Borman, Stuart, and Wheeler (1944) will be used.

METHODS OF INVESTIGATION

The native Arab population of this area, living as it does under primitive sanitary conditions, is obviously the reservoir of intestinal pathogens. It was desirable, therefore, in order to determine what organisms were normally present, to obtain feces specimens from this group of people. This was done through the co-operation of a local Arab hospital, which furnished specimens from newly admitted patients with disorders other than intestinal ones. As a further control on normally occurring organisms, specimens from healthy American soldiers or from Italian prisoner of war food handlers, living under Army sanitary conditions, were obtained. Specimens from these sources were collected during the same period that specimens from hospital patients with enteritis were examined.

All specimens were planted on eosin methylene blue agar, SS agar, and selenite-
f broth immediately on arrival. After 24 hours' incubation, suspicious colonies on the plates were transferred to Kligler agar slants. Material from the selenite-f broth was planted on SS agar, and colorless colonies were picked the following day. All cultures so obtained were tested biochemically. Commonly accepted pathogens were identified serologically, and preliminary studies on the antigenic relationships of the most frequently occurring *Paracolobactrum* species were made.

**DISTRIBUTION OF ORGANISMS**

Table 1 shows the distribution of the most frequently isolated slow- and non-lactose-fermenting organisms obtained. As was expected, the greatest number of positive isolations was made from hospital cases, amounting to 81.1 per cent of the hospital specimens submitted, as compared to 61.1 per cent positive normal Arab specimens, and 32.6 per cent positive normal American specimens.

*Salmonella* species were isolated from 13.5 per cent of the hospital specimens, from 5.5 per cent of normal Arab specimens, and from 8.7 per cent of normal American specimens. *Shigella* species were isolated from only 8.1 per cent of hospital specimens, from 18.5 per cent of normal Arab specimens, and from 4.3 per cent of normal American specimens. Though these figures indicate an apparent lack of significance in the distribution of the commonly accepted pathogens, there can be little question that *Salmonella* and *Shigella* species isolated from hospital patients were in fact the causes of the enteric infections. Most of the positive hospital stools were pathological, containing blood or mucus or both, though some were of a more normal appearance. *Paracolobactrum* species were isolated in only a few cases in association with the ordinary pathogens. The high percentage of *Salmonella* and *Shigella* species isolated from normal American and normal Arab specimens, whose stools in most cases were normal in appearance, indicates a high frequency of the carrier state, a condition which could be expected in a highly endemic area.

There was, on the other hand, a marked difference in the distribution of *Paracolobactrum* species isolated from the three sources. Organisms of that group were obtained from 62.2 per cent of hospital specimens, from 42.6 per cent of normal Arab specimens, and from 19.5 per cent of normal American specimens.

In addition to the organisms recorded in Table 1, anaerogenic organisms possibly belonging to the genus *Proshigella* were isolated but were not of significance either in numbers or distribution. Numerous *Proteus* species were also isolated, being found in 29.6 per cent of normal Arab specimens, in 10.8 per cent of hospital specimens, and in 2.2 per cent of normal American specimens.

Table 2 shows the distribution of the *Paracolobactrum* cultures obtained. *P. aerogenoides* cultures were found about five times as often in hospital patients as in normal Arabs, and about seven times as often as in normal Americans. *P. intermedium* cultures were isolated about twice as often from hospital patients as from normal Arabs, and about three times as often as from normal Americans. *P. coliforme*, however, was isolated only twice as often from hospital patients as from normal Americans, and only half as often as from normal Arabs.
PATHOGENICITY OF PARACOLOBACTRUM SPECIES

CULTURAL AND ANTIGENIC CHARACTERISTICS

Cultural characteristics and antigenic relationships of the Paracolobactrum cultures isolated are shown in table 3. Most of the cultures were held on nonfermentable carbohydrate media, citrate agar (Simmons), and urea agar (Christensen, 1946) for 3 weeks in the preliminary studies. Hydrogen sulfide production was determined in Kligler's medium. Indole production was determined by means of Kovac's reagent, and acetylmethylcarbinol production, because of the lack of more sensitive reagents, was determined by use of 10 per cent potassium hydroxide solution. Semisolid agar at room temperature and at 37°C was used to determine motility. Following the preliminary study, made immediately upon isolation of the cultures, all reactions were repeated and additional tests were added. Most cultures were held on nonfermentable carbohydrate broths in the second series for 40 days.

For antigenic studies, one strain from each of the largest cultural groups was used for antiserum production. This study was to have included complete antigenic comparison among themselves of the Paracolobactrum organisms isolated, as well as comparison with known Salmonella and Shigella antigens. The investigation was terminated, however, before complete antigenic and cultural studies were completed. It will be noted that cultural characteristics of only 20 P. coliforme organisms are given in table 3, though a total of 26 cultures were isolated, as shown by their IMViC reactions. Lack of time prevented more complete study of the 6 cultures not listed, and also of cultures listed which were not tested on all media given in the table.

### Table 1
Comparative distribution of Paracolobactrum, Salmonella, and Shigella species

<table>
<thead>
<tr>
<th>SOURCE OF SPECIMEN</th>
<th>NUMBER OF SPECIMENS</th>
<th>POSITIVE SPECIMENS</th>
<th>SPECIMENS CONTAINING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Per cent</td>
<td>No.</td>
</tr>
<tr>
<td>Hospital patients</td>
<td>37</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Normal Arabs</td>
<td>54</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>Normal Americans</td>
<td>46</td>
<td></td>
<td>15</td>
</tr>
</tbody>
</table>

### Table 2
Distribution of Paracolobactrum cultures

<table>
<thead>
<tr>
<th>SOURCE OF CULTURE</th>
<th>P. AEROGENOIDES</th>
<th>P. INTERMEDIUM</th>
<th>P. COLIFORME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Per cent</td>
<td>No.</td>
</tr>
<tr>
<td>Hospital patients</td>
<td>11</td>
<td>29.7</td>
<td>7</td>
</tr>
<tr>
<td>Normal Arabs</td>
<td>3</td>
<td>5.6</td>
<td>5</td>
</tr>
<tr>
<td>Normal Americans</td>
<td>2</td>
<td>4.1</td>
<td>3</td>
</tr>
</tbody>
</table>

Percentages are in terms of number of specimens examined. See table 1.
### TABLE 3

Cultural characteristics and antigenic relationships of *Paracoloebactrum* cultures

<table>
<thead>
<tr>
<th>ORGANISMS</th>
<th>GROUP</th>
<th>NO. OF STRAINS</th>
<th>H₂S</th>
<th>INDOLE</th>
<th>V. P.</th>
<th>CITRATE</th>
<th>UREA</th>
<th>GELATIN</th>
<th>MOBILITY</th>
<th>GLUCOSE</th>
<th>MANNOITOL</th>
<th>MALTOSE</th>
<th>LACTOSE</th>
<th>SUCROSE</th>
<th>SALICIN</th>
<th>NO. OF CULTURES ANTI-GENICALLY RELATED</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aerogenoides</em></td>
<td>1</td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>9+</td>
<td>15+</td>
<td>-</td>
<td>+</td>
<td>2-8 days</td>
<td>+</td>
<td>7+</td>
<td>2-3 days 1- 8-0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1+</td>
<td>2-</td>
<td>8-1</td>
<td>11+</td>
<td>+</td>
<td>+</td>
<td>4-7 days</td>
<td>+</td>
<td>- or + 11-20 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>1+</td>
<td>-</td>
<td>±</td>
<td>9-35 days</td>
<td>±</td>
<td>- or + 9-35 days</td>
<td>-</td>
<td>- or + 6 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ = positive reaction; acid and gas in carbohydrate broth. ± = acid only or acid and bubble of gas in carbohydrate broth; some strains motile, others nonmotile, others not tested. — = negative reaction; carbohydrate not fermented in 40 days. -0 = not tested.
Only one cultural group of *P. aerogenoides* was isolated in this study. This group is apparently the same as type 1721 of Stuart *et al.* (1943). Of the 16 cultures in this group, 13 were antigenically related, as shown by spot plate and tube agglutination tests. The 3 unrelated strains were all from hospital patients. The antigenic relationship of the other 13 strains was not a close one, however, since adsorption of the serum with the heterologous strains failed to reduce the titer for the homologous organism.

Two cultural groups of *P. intermedium* were found. Group 1 resemble most closely the type 1421 of Stuart *et al.* (1943), with the differences that all cultures studied produced hydrogen sulfide, 2 of the 12 strains produced acid and gas in salicin in 11 and 20 days, and all were definitely aerogenic with all carbohydrates fermented. Of this cultural type, 7 were isolated from hospital patients, 5 from normal Arabs, and none from normal Americans. Antigenically, 4 of the 7 strains which were tested against one antiserum were related. The 4 related strains were from normal Arabs, and the other 3 were from hospital patients. Adsorption experiments were not conducted. Group 2 strains were not held on citrate medium more than 1 week, and it is possible that longer incubation would have brought out citrate utilization. These cultures differed from group 1 in being hydrogen-sulfide-negative and negative or slow on sucrose. All organisms of this group were isolated from normal Americans.

Three cultural groups of *P. coliforme* were isolated. The 9 cultures of group 1 were negative on both lactose and sucrose after 3 weeks in 1 per cent and 40 days in 5 per cent concentrations of the sugars. Six of the 9 were positive in salicin after 2 to 4 days. Some of the cultures were also tested on xylose and inositol. Three of the cultures were negative on xylose, 5 produced acid and gas immediately, and 1 culture was positive after 3 days. Eight cultures tested on inositol were all negative. Antigenically, only 2 of the 9 cultures were related. Adsorption experiments were not done. Three cultures of this group were from normal Americans and 6 were from normal Arabs.

The 3 cultures of group 2 differed from those in group 1 in fermenting lactose in 9 to 35 days, 2 were negative on salicin, and 1 was positive after 6 days. Two of these cultures were from normal Arabs, and 1 culture was from an individual suffering from a mild diarrhea. The 8 cultures of group 3 were either negative on lactose or produced only acid, or acid and a bubble of gas. All 8 cultures were positive on sucrose. Four cultures of this group were from hospital patients; 3 were from normal Arabs and 1 was from a normal American.

**PATHOGENICITY**

Evidence for the pathogenicity of *Paracolobactrum* species in recent years has been slowly accumulating (Parr, 1939; Weil, 1943). Stuart and Rustigian (1943) presented strong evidence for the pathogenicity of one type of *P. aerogenoides*. Stuart *et al.* (1943) presented further direct evidence for the pathogenicity of *P. aerogenoides* and *P. coliforme* strains. Young (1946) reported three strains of *Paracolobactrum* organisms containing *Salmonella* XXXVIII antigen isolated from patients with clinical symptoms but not from asymptomatic persons.
The present communication adds further evidence for the pathogenicity of Paracolobactrum species. The isolation of these organisms from 60 per cent of gastroenteritis cases as compared to 20 per cent of healthy individuals living under similar (Army) sanitary conditions is at least indicative of pathogenicity, particularly since in only a negligible number of cases were they found in association with commonly accepted pathogens. Although objection may be made to attaching such significance to the distribution of these organisms, particularly in view of the relatively large number of isolations from healthy individuals, it will be observed that relatively large numbers of Salmonella and Shigella species were also isolated from healthy individuals. Furthermore, Neter (1943) has stated that persons with no history of bacillary dysentery may harbor dysentery bacilli. The writer in this investigation also isolated Salmonella cultures from at least one healthy person (himself) with no history of previous Salmonella infection. It would therefore seem quite possible to find pathogenic Paracolobactrum species in healthy persons not susceptible to their invasive capabilities.

It is of further interest, concerning relative distribution of enteric pathogens, that Stuart, Wheeler, and McGann (1946) reported that an antigenically heterogeneous, anaerogenic paracolon organism was isolated almost exclusively from gastroenteritis patients in the vicinity of Providence, Rhode Island, with only 1 strain being isolated from over 300 normal individuals. This same type, however, was isolated frequently from normal individuals in the State of Florida.

Aside from the significance implied by the relative distribution of Paracolobactrum organisms (tables 1 and 2), additional evidence points to the pathogenicity of at least the P. aerogenoides type isolated in this study. In the majority of cases, colonies on the plates were not in pure culture and indeed in many cases were not even very abundant, probably because most specimens were obtained from 3 days to a week after onset of the symptoms. In two cases, however, specimens were obtained within a few hours after the onset of the symptoms. These cases were primarily surgical patients (appendectomy and amputation) suffering from mild diarrhea. Specimens from these patients yielded from few to numerous, apparently identical, colorless colonies on EMB and SS agars, and after streaking to SS agar from selenite-f broth. All cultures so isolated were P. aerogenoides, and no commonly accepted pathogens were found.

In another case, a member of the laboratory staff, who one evening had eaten dinner away from the organization's mess, suffered from a very mild diarrhea the next morning. His stool specimens showed the presence of P. coliforme of group 2, though not in very great abundance. No other pathogens were found. No additional evidence beyond the suggestive distribution was obtained for the pathogenicity of P. intermedium.

Biochemically, the P. coliforme cultures of group 1 are of particular interest. These organisms are apparently identical with the once-designated Salmonella columbensis, recently studied by Fulton (1943). This author studied 18 strains of this biochemical type, the majority of which were obtained from gastroenteritis patients. He felt that this organism, although not belonging to the genus Salmonella, was in fact worthy of not only specific but generic rank.
In the present study, the 9 organisms of this group were all obtained from apparently normal individuals and none from hospital cases. The outstanding characteristic of these organisms, however, was their complete lack of ability to ferment either 1 per cent or 5 per cent lactose and sucrose after 3 weeks' and 40 days' incubation in the respective concentrations. Fulton showed that his 18 strains failed to ferment 5 per cent lactose in 10 days. These reactions do indeed seem to set the organisms apart, and though under the proposed scheme of Borman et al. (1944) they are now included in the P. coli form species, it seems possible that further information will show them to be better delineated as a separate species. It might be added, parenthetically, that of 28 indole-positive strains isolated both before and during this investigation which were negative on lactose and sucrose in 1 per cent concentrations after 1 to 3 weeks, 13 produced acid or acid and gas in 5 per cent of either one or both of the carbohydrates within 1 to 35 days.

Although the fact that the relatively small number of specimens examined in this investigation, together with the relatively small number of organisms of each cultural group isolated, makes it inadvisable to state dogmatically that the Paracolobactrum species studied are pathogenic, it is felt that the evidence presented warrants the conclusion that the P. aerogenoides type is very probably pathogenic in this area (Casablanca, North Africa) and that certain of the cultural groups of P. intermedium and P. coli form are possibly pathogenic. It is felt further that more investigations of the nature herein reported should be made on a larger scale and preferably in the field, with especial attention to the incidence of Paracolobactrum species. Such studies should yield valuable information concerning the general pathogenicity of these organisms, as well as bring to light any geographical differences in the pathogenicity of the cultural types isolated.

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SUMMARY

A study of the comparative distribution of Paracolobactrum, Salmonella, and Shigella species in an area highly endemic for enteric infections showed that Paracolobactrum species were found much more frequently in cases of gastroenteritis than were either of the other two groups. The Paracolobactrum organisms were found with much greater frequency in hospital cases than in normal individuals or two groups—namely, normal Arabs and normal American troops. Salmonella and Shigella species were found with almost equal frequency in all three groups.

Study of the Paracolobactrum species yielded evidence that the one cultural group of P. aerogenoides isolated, consisting of 16 strains, 13 of which were antigenically related, was very probably pathogenic in this area. It was considered
possible that some of the *P. intermedium* and *P. coliforme* strains might be pathogenic.

The significance of the distribution of these organisms in normal individuals in this and other areas is discussed.

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